PHYSIOLOGY OF INSECT DIAPAUSE. XI. CYANIDE-SENSITIVITY OF THE HEARTBEAT OF THE CECROPIA SILKWORM, WITH SPECIAL REFERENCE TO THE ANAEROBIC CAPACITY OF THE HEART [1]

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Among the metabolic changes which accompany the onset of insect diapause is a pronounced decrease in sensitivity to cyanide and carbon monoxide. This fact was first discovered by Bodine and Boell (1934a, 1934b) in diapausing eggs of the grasshopper *Melanoplus,* and has subsequently been studied in further detail in *Melanoplus* (Robbie *et al.,* 1938; Robbie, 1941) and in the Cecropia silkworm. The situation in the case of Cecropia may be summarized as follows.

Cyanide and carbon monoxide are lethal agents for the caterpillar of the Cecropia silkworm—a fact which mirrors the presence in the larval insect of an intact and functional cytochrome system. However, immediately after pupation the cytochrome system undergoes partial breakdown in all tissues except the intersegmental muscles of the abdomen. Simultaneously, the over-all metabolism of the diapausing pupa becomes substantially insensitive to cyanide and high pressures of carbon monoxide. This state of affairs persists throughout the prolonged period of pupal diapause. Finally, months later, the termination of diapause and initiation of adult development are accompanied by re-synthesis of cytochromes and the appearance of a fresh increment of metabolism which is sensitive to carbon monoxide. If one blocks this increment with cyanide or carbon monoxide, the insect's development is brought to a standstill.

On the basis of these findings one may infer that the metabolism during the growing, non-diapausing stages in the life history is mediated by the usual cyanide- and carbon monoxide-sensitive cytochrome oxidase. In this sense there is nothing remarkable about the insect's metabolism before and after the pupal diapause. But what is remarkable is the character of the metabolism of the diapausing insect itself. The clear-cut resistance to cyanide and carbon monoxide suggests that the metabolism of the diapausing insect proceeds *via* some simpler and more primordial system of electron transfer making use of a terminal oxidase other than cytochrome oxidase. Under this point of view, the metabolism of the diapausing pupa is conceived to differ, not only quantitatively, but also qualitatively, from that before and after diapause. This prospect has been examined experimentally by Schneiderman and Williams (1954a, 1954b) and incorporated into a comprehensive theory of the biochemistry of diapause.

Crucial to this interpretation is the breakdown of the cytochrome system at

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the outset of pupal diapause—a matter which has recently been re-examined by Shappirio and Williams (1957a, 1957b) in individual tissues of the Cecropia silkworm. Spectrophotometric studies at room temperature and spectroscopic studies at the temperature of liquid nitrogen confirm that, in all tissues except the intersegmental muscles of the abdomen, rapid changes in the cytochrome system take place at the outset of pupal diapause. Within 24 hours after the pupal molt, cytochromes *b* and *c* decrease at least 30-fold and become indetectable; cytochrome b_5 and cytochrome oxidase ($a + a_3$) likewise decrease at this same time, but then stabilize at low but finite levels. The net effect is that throughout the pupal diapause the tissues contain cytochrome oxidase in large excess over cytochrome *c*. Consequently, if the cyanide- and carbon monoxide-sensitive system fails to participate in the metabolism of the diapausing tissues, then the block in electron transfer must be localized at the level of cytochrome *c* rather than at the level of cytochrome oxidase itself.

Whether cytochrome *c* actually disappears at the outset of diapause is a matter which lies beyond the resolution of the most sensititive methods of assay available at the present time. This is a question of decisive importance because a low concentration of *c* in the presence of a tremendous excess of oxidase might camouflage the participation of the cytochrome oxidase system in the metabolism of diapause. Thus, by means of carbon monoxide or cyanide one could poison, say, 95 per cent of cytochrome oxidase activity and the residual 5 per cent of active oxidase might still be able to saturate cytochrome *c* and sustain the low and "carbon monoxide-insensitive" metabolism of the diapausing insect.

Because of the limitation inherent in methods for the assay of cytochrome *c*, the problem appeared to be intractable to further biochemical analysis at the present time. Therefore, we have directed attention back to the insect itself. We have selected for intensive study the physiology of a particular tissue, the insect heart. Through an investigation of this tissue we have been able to bypass many of the above-mentioned difficulties and to comprehend what appears to be the mechanism of cyanide and carbon monoxide-sensitivity and -insensitivity in the Cecropia silkworm. In the present paper attention is directed to the effects of cyanide on the heartbeat of the insect during metamorphosis. In the following paper (Harvey and Williams, 1958) the effects of carbon monoxide will be considered.

Materials and Methods

1. *Experimental animals*

The experimental animals, *Platysamia cecropia* L., were reared and managed according to methods described previously (Williams, 1946, 1956). Experiments were performed on the insect at the following stages: mature larvae shortly before the initiation of spinning; unchilled pupae which had been stored at 25° C.; chilled pupae which had been stored at 6° C.; chilled pupae which had been stored for 4 to 6 months at 6° C. and then returned to 25° C. for one week; post-diapausing individuals at successive stages in adult development at 25° C.; and adult moths which had developed and emerged at 25° C. Certain experiments were performed in parallel on the related Polyphemus silkworm (*Telea polyphemus* Cram.).

2. *Methods*

A. *Exposure of isolated hearts to increasing concentrations of cyanide*

The dorsal half of the abdomen was excised with scissors and pinned by its lateral margins to a wax layer in the bottom of a circular dish of Lucite (polymethyl methacrylate). Each dish was provided with a Lucite cover and with inlet and outlet tubes arranged in such a manner that the preparation was automatically bathed in 20 ml. of gently flowing insect Ringer's solution (Ephrussi and Beadle, 1936). The latter was slightly modified by the substitution of 0.001 *M* potassium phosphate buffer (pH 7.0) for a corresponding proportion of the potassium chloride. To the solution prior to use were added a few milligrams of a 1:1 mixture of crystalline phenylthiourea and streptomycin sulfate—the former to block tyrosinase activity and the latter to oppose bacterial growth.

The gut and gonads were removed from the preparation, thereby exposing the heart and alary muscles. The paired masses of fat body were pressed aside so that the heart could be viewed *in situ* through a dissecting microscope.

The physiological solution was aerated continuously by a gentle stream of oxygen introduced into the fluid by a 20-gauge hypodermic needle passing through the lateral wall of the dish. A 26-gauge needle passing through the plastic cover permitted the addition of a solution of hydrogen cyanide; a reservoir of the latter was stored in a one-liter Pyrex wash bottle which was connected by Tygon tubing to the hypodermic needle.

The preparation was first equilibrated with insect Ringer until the heartbeat was stabilized. This ordinarily required one to two hours. The flow of Ringer was stopped and the cyanide solution was then dripped into the perfusion fluid at a rate of approximately ten drops per minute. The concentration of cyanide in this stock solution was 10 to 100 times the inhibitory level, as ascertained in preliminary experiments. The dropwise addition of cyanide was continued until the heartbeat was strongly inhibited. The oxygen flow was shut off and a two-ml. sample of the perfusion fluid was then immediately withdrawn into a hypodermic syringe and analyzed for cyanide by the phenolphthalin technique described by Robbie (1944).

B. *Exposure of isolated hearts in a flowing system*

An elongate plastic tube, 1.9 cm. in outside diameter, was cut longitudinally to form two semi-cylindrical troughs. The depression was then filled with melted wax. A series of hearts was isolated and pinned to the wax bottom of the plastic trough; the latter was then slipped into a glass tube (60 cm. long and I. D. 2 cm.). The glass tube was equipped with ground glass joints at its two ends. One end was connected by the ground joint to a stoppered reservoir containing the solution to be tested. The latter was forced from the reservoir by a slight positive pressure of overlying oxygen or nitrogen. The solution, after flowing slowly over the abdomens, made exit from the ground joint at the distal end of the glass tube and was passed in rubber tubing into a five-gallon bottle containing strong alkali. As the occasion required, samples of solution were withdrawn from the rubber tube with a hypodermic syringe and analyzed for cyanide or for oxygen.

C. *Appraisal of heartbeat*

In Method A the constant agitation of the oxygen bubbles caused considerable irregularity in the frequency of beat. Therefore, in appraising the heartbeat in experiments utilizing Method A, primary attention was centered on the amplitude of the beat rather than its frequency. This method of study was soon abandoned in favor of Method B. Here the frequency of heartbeat was found to be far more constant and predictable. Under constant conditions the variability of heart rate was small compared to that brought about by the experimental treatment. Routinely the frequency of heartbeat was counted for each individual over a period of from one to five minutes and averaged as beats per minute. The strength (amplitude) of the beat was also scored as normal (3), subnormal (2), barely detectable (1), and absent (0). In order to obtain an over-all index of heart function, the recorded frequencies were divided by 1 when the heartbeat was normal, by 2 when the beat was subnormal, and by 3 when the beat was barely detectable. We shall hereafter refer to this value as the "heartbeat index."

D. *Reagents*

Cyanide was obtained as potassium cyanide (Mallinckrodt) assaying not less than 96.0%. Fresh solutions were prepared daily in oxygenated Ringer, neutralized with 1 N hydrochloric acid to pH 7.0, and stored in stoppered containers in the cold. At this pH, 98% of the cyanide is present as hydrocyanic acid.

The experimental gases were obtained in pressure cylinders and assayed as follows: "pre-purified nitrogen" (Airco), 99.998%; oxygen (Airco), 99.5%.

Results

1. *Acute poisoning of the isolated heart*

Isolated hearts of Cecropia, at successive stages in metamorphosis, were exposed during a period of one-half hour to increasing concentrations of cyanide by Method A. The concentration required to inhibit the heartbeat during this half hour was ascertained for each of a series of animals at each of seven stages in metamorphosis. When judged in this manner, the cyanide-sensitivity of the heartbeat is found to undergo large and systematic changes during the course of metamorphosis.

As recorded in Table I and Figure 1, the heartbeat of the mature larva is blocked within 0.5 hour by cyanide concentrations somewhat less than 10^{-3} *M*. However, immediately after the pupal molt, a remarkable resistance to cyanide becomes evident. Thus, within one day after the molt, the inhibitory cyanide concentration increases to 5×10^{-3} *M*. This trend continues until, some two to three weeks later, the inhibitory concentration is not far short of 10^{-1} *M*. The net effect is that the transition of the larva into a diapausing pupa is accompanied by a 100-fold decrease in sensitivity to acute poisoning by cyanide. This condition then persists during the months of pupal diapause.

After prolonged exposure to 6° C., the pupal diapause is terminated; only one or two days at 25° C. are then required for the visible initiation of adult development (Williams, 1956). Though pupae of this type show no detectable development when examined immediately after their return to 25° C., it is of interest that

resistance to cyanide has already begun to decline (Table I and Fig. 1). By the first or second day of adult development the heart is approximately as sensitive to cyanide as the larval heart. During the three-week period of adult development at 25° C., one records an ever-increasing sensitivity to acute poisoning by cyanide. Finally, the heart of the freshly emerged adult moth is blocked by cyanide at concentrations as low as 10^{-5} *M*—a sensitivity 8,000 times that recorded for the diapausing pupa.

TABLE I

Acute toxicity of cyanide for Cecropia and Polyphemus hearts: cyanide concentrations which block the heartbeat during 0.5-hour exposure*

P. cecropia

Stage	No. of preparations	Final concentration of cyanide ($\times 10^{-4}$ *M*)	Reversibility of effects
Fifth instar larva	7	7.5 ± 0.46**	+
One-day-old pupa	6	50.0 ± 4.80	0
Pupa after 2–3 weeks at 25° C.	6	770.0 ± 170.00	0
Pupa after 8 months at 6° C.	12	77.0 ± 6.90	0
First or second day of adult development at 25° C.	5	5.1 ± 1.20	+
Fifteenth or sixteenth day of adult development at 25° C.	6	3.0 ± 1.70	+
Adult moth	21	0.1 ± 0.04	+

T. polyphemus

Stage	No. of preparations	Final concentration of cyanide ($\times 10^{-4}$ *M*)	Reversibility of effects
Pupa after 5 months at 25° C.	6	350.0 ± 44.00	0
Pupa after 7 months at 6° C.	11	31.0 ± 8.30	0
Eleventh or twelfth day of adult development at 25° C.	4	12.0 ± 3.10	0
Adult moth	8	0.5 ± 0.21	+

* No beat or only trace of beat during one minute of observation.
** Standard error.

As summarized in Table I, the observations were repeated on pupae and adults of the Polyphemus silkworm (*Telea polyphemus*). Here again, the cyanide resistance of the pupal heart is evident.

For both these species the response of the pupal hearts to acute poisoning by cyanide is remarkable, not only in terms of the high concentrations required to inhibit the heartbeat, but also in terms of the irreversibility of this inhibition (Table I). Whereas the heartbeat of larvae, developing adults, and adults is promptly re-

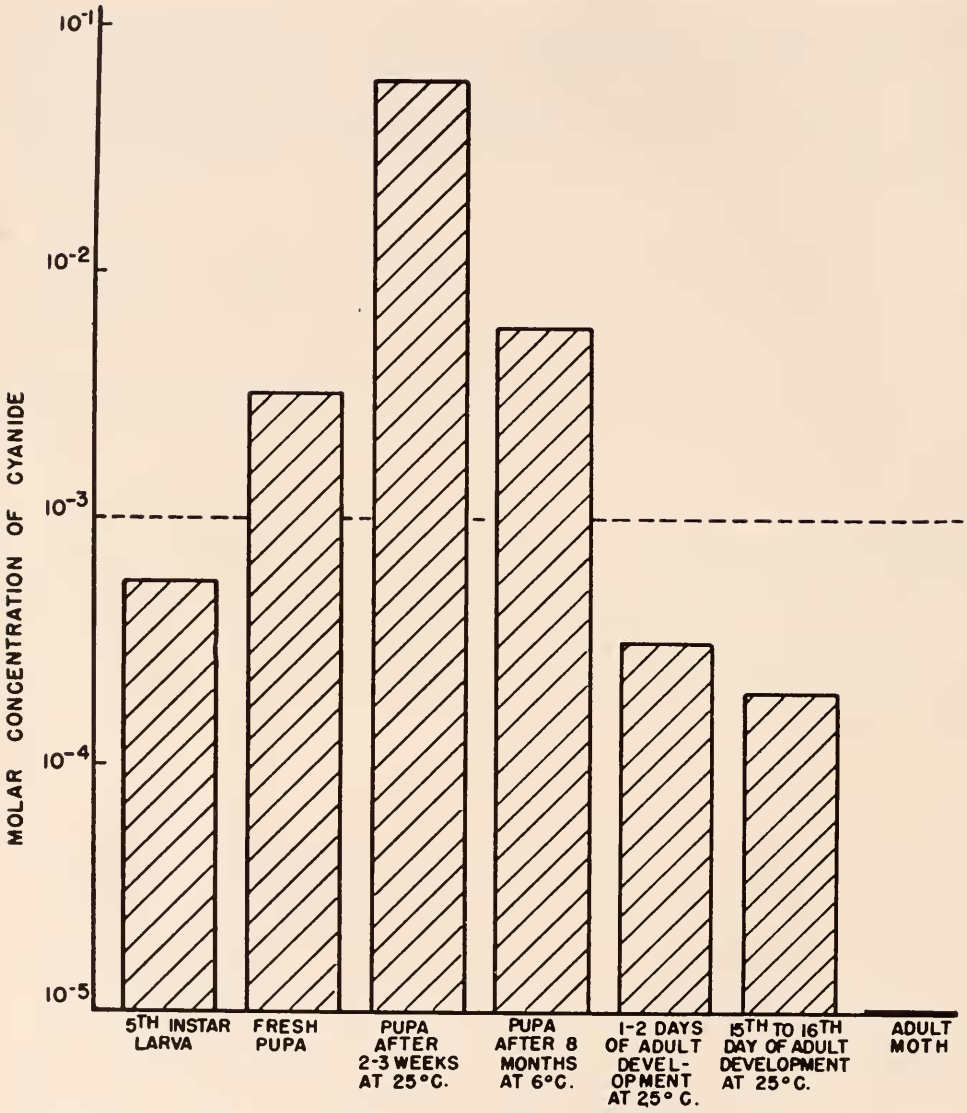

FIGURE 1. Cyanide concentrations required to block the beat of the isolated heart of the Cecropia silkworm within 0.5 hour. The resistance of the heart to acute cyanide-poisoning is seen to undergo major changes during the larval-pupal-adult transformation.

gained when returned to cyanide-free Ringer, the pupal hearts are evidently killed by the high concentrations required to inhibit them.

The experiment therefore directs attention to the paradoxical behavior of the pupal heart in relation to poisoning by cyanide. As illustrated in Figure 1, the pupal heart continues to beat normally for at least a half hour in cyanide concentrations far exceeding 10^{-3} M; that is, under conditions where one would anticipate

the inhibition of the vast majority of cytochrome oxidase activity. How can one account for this resistance of the pupal heart to cyanide?

One possibility is that the pupal heart contains a terminal oxidase other than cytochrome oxidase, and that this unknown oxidase is insensitive to cyanide. However, it was necessary to consider an even simpler explanation; namely, that the pupal heartbeat can be sustained by strictly anaerobic processes.

2. *Pupal heartbeat under anaerobic conditions*

The hearts of four diapausing pupae were isolated and pinned in the bottom of the glass tube described under Method B above. The tube was first perfused with a gently flowing stream of oxygenated Ringer, and the heartbeat of each individual ascertained. The 400-ml. tube was then perfused rapidly with oxygen-free insect Ringer; the perfusion was then continued at the lower rate of approximately 500 ml. per hour. Special attention was given to the total removal of oxygen from the physiological solution prior to its use. For this purpose, pre-purified nitrogen was bubbled through the Ringer for at least two hours; moreover, after traversing the solution the nitrogen was bubbled through a solution of reduced methylene blue (Fildes, 1931). The absence of color change gave assurance that all oxygen had been removed from the Ringer. The latter was then stored under a slight positive pressure of pre-purified nitrogen, and displaced by this pressure through the tube containing the hearts.

The hearts of four diapausing pupae were studied—first in air, then for 5½ hours in oxygen-free Ringer, and, finally, for 43 hours in oxygenated Ringer. The various measurements are summarized in Table II, along with the average heartbeat indices.

One is immediately impressed by the striking resistance of these diapausing hearts to strictly anaerobic conditions. After 0.5 hour of anaerobiosis, none of the hearts showed any detectable depression. After one hour, only one of the four was depressed. Between the first and second hours the over-all index value decreased

TABLE II

Effects of strictly anaerobic conditions on isolated hearts of brainless diapausing Cecropia pupae

Animal No.	Rate (beats/min.) and amplitude* of heartbeat										
	Air	Hours in Ringer equilibrated with pre-purified nitrogen						Subsequent hours in oxygenated Ringer			
		½	1	2	3	4½	5½	¼	1½	25	43
1	12 (3)	17 (3)	1 (1)	0 (0)	0 (0)	0 (0)	0 (0)	8 (3)	22 (3)	17 (3)	10 (3)
2	13 (3)	13 (3)	12 (3)	12 (2)	13 (3)	5 (3)	4 (2)	14 (3)	17 (3)	13 (3)	9 (3)
3	17 (3)	19 (3)	19 (3)	13 (2)	0 (0)	14 (2)	0 (0)	20 (3)	4 (3)	6 (3)	7 (3)
4	12 (3)	20 (3)	19 (3)	14 (3)	12 (2)	8 (1)	0 (0)	26 (3)	19 (3)	13 (3)	8 (3)
Average heartbeat index	13.5	17.2	12.5	6.8	4.8	3.8	0.5	17.0	15.5	12.2	8.5

* See Methods.

markedly; however, one of the hearts showed a normal beat after two hours of anaerobiosis and another, after three hours. Three of the four hearts were still beating after 4½ hours of anaerobiosis, and one, after 5½ hours.

It will be observed in Table II that the effects of 5½ hours of anaerobiosis were promptly reversed when the hearts were returned to oxygenated Ringer. Indeed, the average index values actually increased for about an hour over the level at the

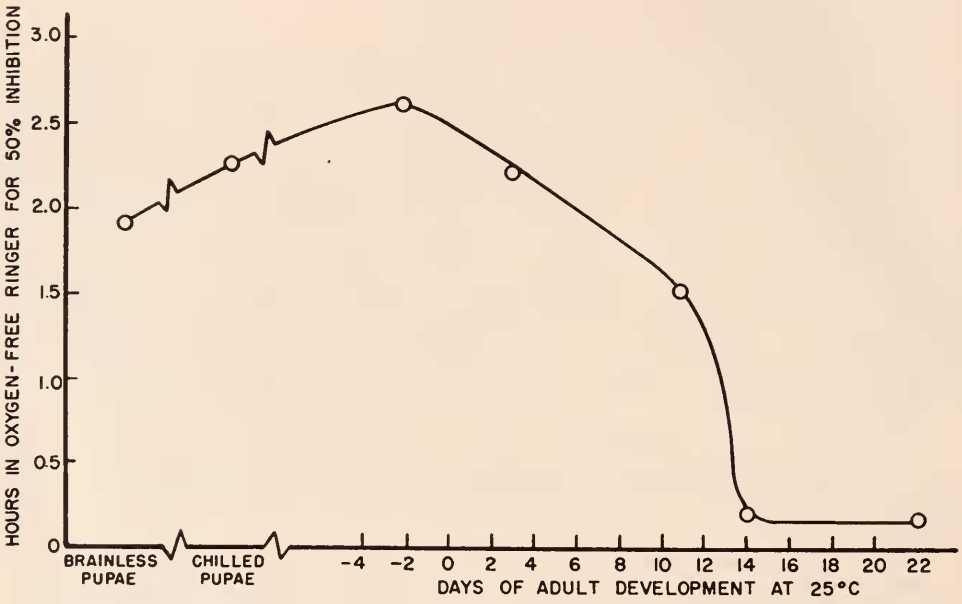

FIGURE 2. The "anaerobic reserve" of the isolated Cecropia heart is plotted as a function of pupal-adult development. The discontinuities in the curve correspond to days or weeks of storage under the conditions noted on the X-axis. The anaerobic reserve declines almost to zero during the course of adult development. Each datum is the average derived from the hearts of four to eleven individuals.

outset, and then stabilized at or near the initial, normal level. The absence of any permanent damage attributable to anaerobiosis is also confirmed by the continuation of heartbeat for 1½ further days until the experiment was abandoned.

3. *Heartbeat of chilled pupae, developing adults, and adults under anaerobic conditions*

Is the high degree of facultative anaerobism peculiar to the heart of the diapausing pupa? In order to answer this question the experiment, just considered, was repeated on the hearts of: previously chilled pupae; chilled pupae that had been returned to 25° C. and were just prior to the initiation of adult development; developing adults; and adults. The results are recorded in Figure 2 in terms of the period of anaerobiosis required for the reversible inhibition of 50 per cent of the average heartbeat index. The method of arriving at this value is illustrated in Figure 3. The average heartbeat indices of the diapausing pupae are here plotted

as a function of the duration of exposure to oxygen-free Ringer. A smooth curve is drawn by inspection through the series of points and the time for 50 per cent inhibition is ascertained from the curve.

In the results summarized in Figure 2, it is clear that a considerable capacity for anaerobism persists within the pupa during the months of chilling at 6° C. When pupae of this type are placed at room temperature, the capacity for anaerobism actually appears to increase slightly. By the fourth day of adult development the "anaerobic capacity" has returned to the level observed in diapausing pupae. This trend continues and by the eleventh day of adult development the time for 50 per cent inhibition under anaerobic conditions has dropped to 1.5 hours. On or about the eleventh day of adult development the anaerobic capacity decreases precipitously to the low level characteristic of the adult moth. The adult moth, emerging after 21 days of development at 25° C., is maximally sensitive to oxygen lack in that the heart is able to beat less than 10 minutes in the total absence of oxygen.

In the experiments just considered, the anaerobic condition was established by the use of oxygen-free Ringer's solution. The observations on pupal and adult hearts were repeated in a series of experiments in which the anaerobic condition was established by the ventilation of the tube with a flowing stream of pre-purified nitrogen gas (300 ml. per hour). Precisely the same results were observed.

In a further series of experiments making use of pre-purified nitrogen, the findings were confirmed in studies of the pupal and adult hearts of *Telea polyphemus*.

Consequently, for both these species, it is clear that the pupal heart, unlike the adult heart, possesses a substantial "anaerobic reserve" which can sustain the beating of the heart for as long as 5½ hours in the total absence of oxygen. Aside from the intrinsic interest of this new finding, the anaerobic capacity of the pupal heart is obviously critical in the design of experiments testing the aerobic metabolism of the pupal heart.

4. *Sensitivity of the pupal heart to prolonged exposure to cyanide*

In Section 1 the pupal heart was found to be extremely resistant to cyanide. However, it will be recalled that this result was based on experiments of short duration in which the heart was exposed to increasing concentrations of cyanide during a period of 0.5 hour. We now see that the pupal heart can beat for up to 5½ hours in the total absence of oxygen. The earlier experiments were therefore inadequate as a test of the cyanide sensitivity of the pupal heart. For this reason the effects of cyanide on the pupal heartbeat were re-examined in experiments of prolonged duration.

Isolated pupal hearts were placed in the flow tube (Method B) and subjected to a flowing stream of oxygenated insect Ringer containing a precise concentration of cyanide. The reservoir of Ringer was prepared in a stoppered five-gallon bottle and stored under oxygen. In order to cause the Ringer to flow through the experimental tube, the reservoir was slightly compressed by the addition of a stream of oxygen; the latter was bubbled through an aqueous solution of 10^{-1} or 10^{-2} *M* potassium cyanide before entering the reservoir. In this manner it was possible to prevent any significant change in the cyanide concentration in the Ringer during prolonged experiments. This fact was confirmed by cyanide assays performed on fluid that had traversed the chamber.

Cyanide at two specific final concentrations was studied in detail; namely, 10^{-2} *M* and 10^{-3} *M*. It will be recalled that both these concentrations were without detectable effects on the pupal heartbeat in experiments of short duration.

The effects of 10^{-2} *M* cyanide are summarized in Figure 4. In terms of the average index values, the heartbeat remained normal for 1½ hours. Two of the six hearts stopped beating after 2 hours. After 3½ hours, all hearts showed considerable depression, and three of the six had stopped. The average time required for 50 per cent inhibition was 2.25 hours. The tube was then flushed with cyanide-free Ringer. Three hearts showed a slight recovery at this time. This was a temporary effect, however, for all six hearts were in standstill after a total of two hours in cyanide-free Ringer.

In like manner the effects of perfusion with oxygenated Ringer containing 10^{-3} *M* cyanide were studied. A considerable depression was first evident after 2¾ hours, but all four hearts were still beating after 4 hours. At the end of 5¼ hours, two of the hearts stopped beating and the other two showed only a trace of beat. At this time the system was flushed with cyanide-free Ringer. All four hearts showed a delayed recovery and three of the four were beating normally after a total of 16 hours in cyanide-free Ringer.

Discussion

The heartbeat of the larva and the adult Cecropia silkworm is blocked in a reversible manner by brief exposure to cyanide in concentrations less than thousandth molar. Therefore, on the basis of this classical test, it seems safe to conclude that the hearts of the larva and the adult moth make use of cytochrome oxidase as "terminal oxidase."

When the same criterion is applied to the pupal heart, the latter is found to beat normally when immersed in cyanide at concentrations not far short of tenth molar. Here, then, is a tissue which appears to be totally insensitive to cyanide over the range of concentrations at which cyanide is an inhibitor of cytochrome oxidase. Consequently, the pupal heart has appeared to be a clear instance of a cyanide-insensitive tissue whose function is not dependent on metabolism mediated by cytochrome oxidase. Prior to the present investigation, we have routinely thought that this was so (Williams, 1951; Harvey and Williams, 1953).

On the basis of the experimental results described above, it is now clear that the pupal heart is by no means insensitive to cyanide. The crux of the matter is that the true cyanide-sensitivity of the heart is camouflaged most effectively by an anaerobic capacity peculiar to the pupal heart. Whereas the adult heart is able to beat for less than ten minutes in the total absence of oxygen, the pupal heart, by contrast, beats normally for one or more hours under the same conditions.

The experimental conditions leave little room for doubt that the pupal heartbeat, during this prolonged period of facultative anaerobism, is sustained by strictly anaerobic metabolism. Under this circumstance the heart can scarcely require the function of cytochrome oxidase or, for that matter, any other enzyme concerned with the utilization of atmospheric oxygen. Therefore, for a corresponding period the pupal heart is found to be totally insensitive to physiological concentrations of cyanide.

The true sensitivity of the pupal heart to cyanide is unmasked only when one

continues the experiment sufficiently long to use up the anaerobic reserve. This fact is evident in a comparison of Figures 3 and 4. The inhibition of the pupal heart by cyanide (Fig. 4) shows precisely the same time-course as that observed under anaerobic conditions in the absence of cyanide (Fig. 3). As the heart exhausts its anaerobic reserve, it becomes progressively more dependent on aerobic metabolism and progressively more sensitive to cyanide. These observations strongly argue that the aerobic metabolism of the diapausing heart requires the presence and function of cytochrome oxidase.

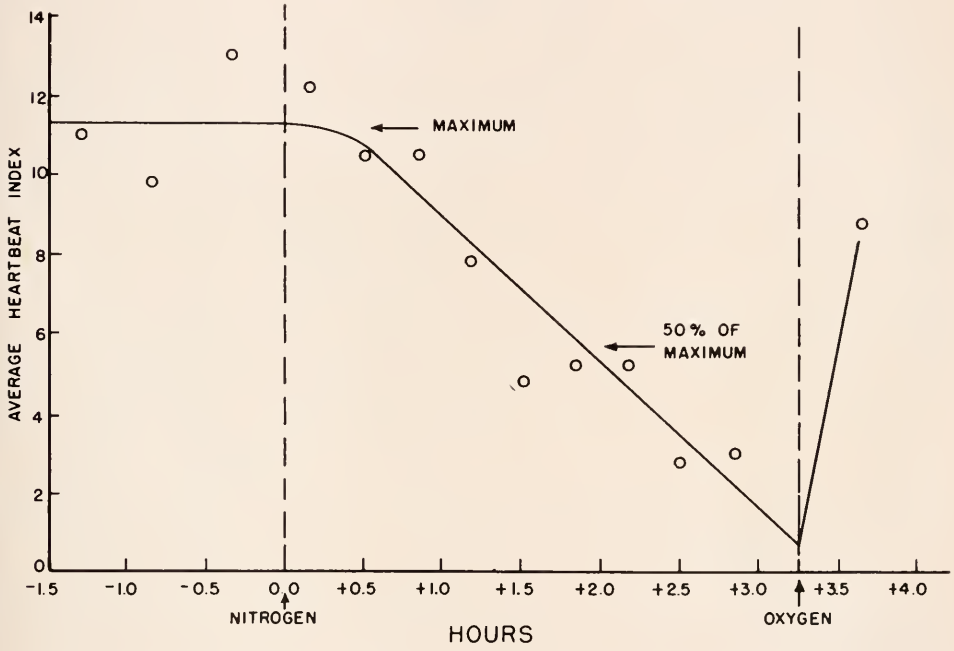

FIGURE 3. Technique for determining the time required for the 50 per cent inhibition of the heartbeat index during exposure of isolated hearts to oxygen-free Ringer. Each datum is the average from the hearts of eight brainless diapausing pupae.

On the basis of our present data we are unable to state the lower limit of cyanide concentration which inhibits the pupal heart in experiments of this type. However, for reasons which will be considered in detail in the following paper, we doubt that the pupal heart can ever be inhibited by the very low cyanide concentrations (10^{-5} *M*) which suffice to block the heartbeat of the adult moth.

While clarifying the problem of the cyanide-insensitivity of the pupal heart, the present study directs attention to a fresh problem—the changes occurring in the insect's capacity for anaerobic metabolism during the course of metamorphosis. As illustrated in Figure 2, these changes are large and systematic. Of particular interest is the rapid loss of "anaerobic reserve" which supervenes approximately midway in adult development.

We suspect that this change is not peculiar to the heart. Thus, according to Schneiderman and Williams (1954b), mature larvae and adult moths of the Ce-

cropia silkworm are killed in less than one day when exposed to "tank nitrogen" containing less than 0.5% oxygen. Diapausing pupae, by contrast, survive more prolonged exposures, the L.D. 50 per cent being three days.

On the basis of present inadequate information we are unable to comprehend the full meaning of this shift in the capacity for anaerobic metabolism. The quantita-

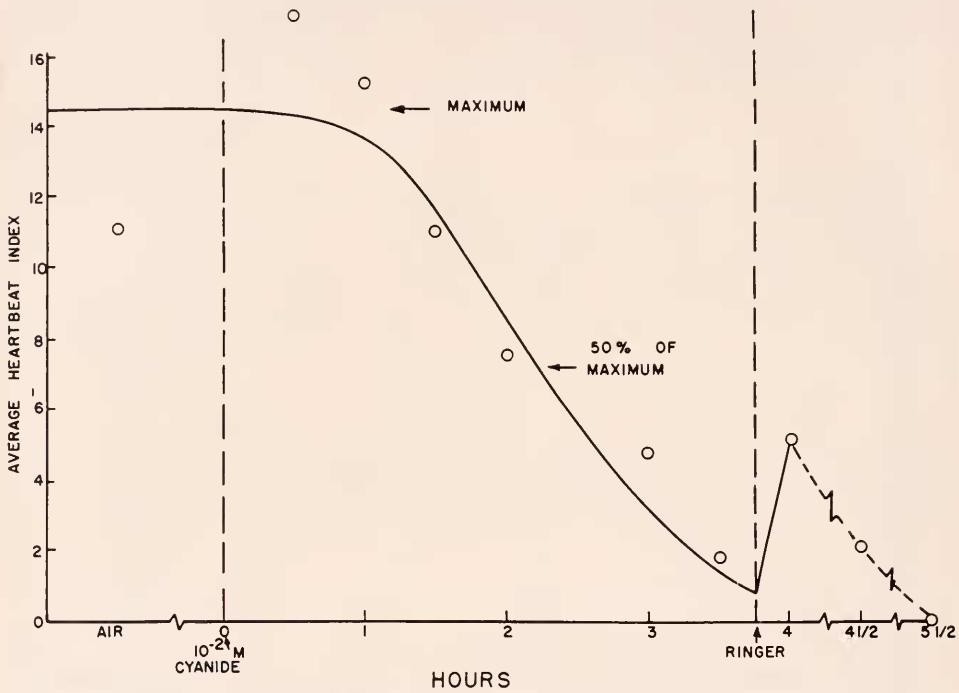

FIGURE 4. Effects of 0.01 *M* cyanide on the beat of hearts isolated from diapausing Cecropia pupae. Each datum is the average derived from the hearts of six brainless diapausing pupae.

tive changes suggested in Figure 2 are almost precisely the reverse of those occurring in the over-all aerobic metabolism and in the concentration of such typically aerobic enzymes as the cytochromes. The problem obviously merits further study.

SUMMARY

1. During the course of metamorphosis the heart of the Cecropia silkworm appears to undergo pronounced shifts in its sensitivity to cyanide.

2. In the mature larva the heartbeat is promptly blocked by 10^{-3} *M* cyanide; in the adult moth it is even more sensitive and is brought to a standstill by 10^{-5} *M* cyanide.

3. In the intervening pupal stage the heart is insensitive to acute poisoning by physiological concentrations of cyanide.

4. This insensitivity is observed only in experiments of short duration. When the exposure to cyanide is continued for many hours, the pupal heartbeat is blocked by 10^{-2} or 10^{-3} *M* cyanide.

5. The paradoxical response of the pupal heart can be accounted for in terms of a pronounced capacity for anaerobic metabolism which is peculiar to this particular stage. The pupal heart can beat for as long as 5½ hours in the complete absence of oxygen. During this same period the heart is insensitive to cyanide.

6. While discounting any true insensitivity of the pupal heart to cyanide, the experimental results direct attention to major and previously unsuspected changes in the anaerobic capacity of the Cecropia silkworm during the course of metamorphosis.

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